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Dose Separation Does Not Overcome the Pharmacokinetic Interaction between Fosamprenavir and Lopinavir/Ritonavir

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Previous investigations have shown a significant negative two-way drug interaction between fosamprenavir (FPV) and lopinavir/ritonavir (LPV/RTV) in both human immunodeficiency virus (HIV)-infected patients and seronegative volunteers. This randomized, nonblinded, three-way crossover study of HIV-seronegative adult volunteers investigated dose separation and increased doses of RTV as a means to overcome the interaction between FPV and LPV/RTV. Eleven HIV-seronegative volunteers were given FPV plus LPV/RTV at 700 mg plus 400/100 mg every 12 hours (q12h) simultaneously for 10 days and then randomized to receive each of three 7-day treatments in one of six possible sequences, as follows: FPV plus LPV/RTV at 700 mg plus 400 mg/100 mg q12h simultaneously, FPV/RTV at 700 mg/100 mg q12h plus LPV/RTV at 400 mg/100 mg q12h, with doses separated by 4 h, and FPV/RTV at 1,400 mg/200 mg in the morning plus LPV/RTV at 800 mg/200 mg in the evening. Pharmacokinetic sampling was performed on day 8 of each treatment, and samples were analyzed for FPV, amprenavir (APV), LPV, and RTV concentrations by high-performance liquid chromatography–tandem mass spectrometry. Geometric mean ratios (GMR [with 95% confidence intervals]) for the 4- and 12-h dose separation strategies compared to simultaneous administration were calculated for the areas under the concentration-time curves from 0 to 24 h. Compared to simultaneous administration, RTV exposures increased with both 4-h and 12-h dose separation strategies (GMR, 5.30 [3.66 to 7.67] and 4.45 [3.09 to 6.41], respectively). LPV exposures also significantly increased with both 4-h and 12-h dose separation strategies (GMR, 1.76 [1.34 to 2.32] and 1.43 [1.02 to 2.01], respectively). However, both the 4- and 12-h strategies resulted in greater reductions in APV exposure (0.67 [0.54 to 0.83] and 0.77 [0.59 to 0.99], respectively) compared to simultaneous administration. Additional investigations are warranted to determine the optimal dosing of FPV with LPV/RTV.

As larger numbers of antiretroviral-experienced human immunodeficiency virus (HIV)-infected patients have fewer options for therapy, novel combinations of potentially synergistic and/or complementary antiretrovirals are increasingly being evaluated. The use of two virologically active protease inhibitors combined with low-dose ritonavir is one such strategy (8, 11, 16, 17, 21, 22, 24, 25, 31). Clinical and pharmacokinetic data for a combination of the capsule formulation of amprenavir (APV) (Agenerase; GlaxoSmithKline, Research Triangle Park, NC) and lopinavir/ritonavir (LPV/RTV) (Kaletra; Abbott Laboratories, Abbott Park, IL) have been published by a number of investigators (4, 9, 13, 19, 20, 26, 31).

In October 2003, fosamprenavir (FPV), the phosphate ester prodrug of amprenavir (Lexiva; GlaxoSmithKline), was approved by the FDA for the treatment of HIV, and as of December 2004, FPV has replaced Agenerase as the currently available form of amprenavir. However, recent investigations have shown that the combination of FPV and LPV/RTV at standard doses results in significantly lower drug exposures than FPV/RTV or LPV/RTV alone (13, 29). In Adult AIDS Clinical Trials Group (ACTG) study A5143, a steady-state pharmacokinetic (PK) evaluation was performed with HIV-infected patients receiving either FPV/RTV, LPV/RTV, or FPV plus LPV/RTV (in combination with tenofovir and one or two additional nucleoside analogue reverse transcriptase inhibitors) (13). Patients receiving this dually boosted combination of protease inhibitors had a 64% decrease in the APV area under the concentration-time curve (AUC) and a 50% decrease in the LPV AUC compared to patients receiving ritonavir-enhanced protease inhibitors. GlaxoSmithKline investigated alternative dosing of FPV/RTV with LPV/RTV in seronegative volunteers (29). The following two regimens resulted in LPV concentrations similar to or slightly higher than those obtained with LPV/RTV at 400/100 mg every 12 h: (i) FPV at 1,400 mg plus LPV/RTV at 533/133 mg twice a day (BID) and (ii) FPV at 700 mg plus LPV/RTV at 400/100 mg plus RTV at 100 mg BID. However, neither of these regimens resulted in APV concentrations similar to those obtained with FPV/RTV at 700/100 mg every 12 h. The regimen of 1,400 mg FPV with 533/133 mg LPV/RTV resulted in APV exposures of 58 to 87% those obtained with FPV/RTV alone, but 31% (11 of 36) of subjects discontinued this regimen due to adverse effects, such as gastrointestinal disturbances, headaches, oral/perioral numbness, pruritis, and rash.

The mechanism of this interaction is unclear. Since increasing doses of FPV and LPV/RTV resulted in a less tolerable regimen and did not fully overcome the drug interaction, additional strategies for coadministration need to be explored.

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Alternative hypotheses for the interaction include the involvement of a physical incompatibility or an acute local interaction involving drug-metabolizing enzymes and/or transporters. Therefore, it was anticipated that separating the administration of FPV and LPV/RTV would result in higher drug exposures than those after simultaneous administration of all three antiretrovirals.

MATERIALS AND METHODS

A prospective, randomized, nonblinded, three-way crossover study of healthy adult HIV-negative volunteers was conducted to compare the PK parameters of FPV, APV, LPV, and RTV when FPV was administered simultaneously with, 4 hours prior to, or 12 hours prior to LPV/RTV.

Healthy volunteers were screened and enrolled if they were 18 to 45 years old, tested HIV seronegative by an enzyme-linked immunosorbent assay, and weighed >50 kg. Subjects were excluded if they had a previous hypersensitivity to APV, LPV, or RTV; were ≥20% over ideal body weight; were pregnant (by positive serum human chorionic gonadotropin); were taking concomitant prescription, nonprescription, herbal, or illicit drugs; were unable to abstain from alcohol or grapefruit products while enrolled; or had any of the following laboratory abnormalities: hematocrit of <30 g/dl; total cholesterol level of >240 mg/dl; triglyceride level of >400 mg/dl; fasting glucose level of >120 mg/dl; alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, or bilirubin level of more than twice the upper limit of normal; or albumin level of <3.5 g/dl. The human experimentation guidelines of the U.S. Department of Health and Human Services and those of the University of North Carolina at Chapel Hill, as well as Health Insurance Portability and Accountability Act regulations, were followed in the conduct of this clinical research study.

Subjects meeting the above criteria were initially given FPV and LPV/RTV simultaneously for 10 days due to the potential drug-metabolizing enzyme-inducing capacities of APV (6, 27) and LPV/RTV (30). However, it was acknowledged that the lower drug exposures with this combination could potentially impact the maximal influence on drug-metabolizing enzyme activity. If the dose separation strategies successfully increased drug exposure, more time would be needed to achieve steady-state conditions. Therefore, after this initial phase, each subject in this prospective, nonblinded, three-treatment, three-period, six-sequence crossover study was assigned by a randomization schedule to one of the six treatment sequences described below (ABC, ACB, BAC, BCA, CAB, and CBA), generated via SAS System software (version 8.2), as summarized in Table 1. The study design and randomization procedure specified a balanced allocation of 12 subjects to the six sequences, i.e., 2 subjects per sequence. On day 11, subjects began the assigned sequences with an initial treatment of either (i) FPV at 700 mg BID plus LPV/RTV at 400/100 mg BID (given simultaneously) (treatment A), (ii) FPV/RTV at 700/100 mg BID plus LPV/RTV at 400/100 mg BID (given 4 h apart) (treatment B), or (iii) FPV/RTV at 1,400/200 mg once a day (QD) plus LPV/RTV at 800/200 mg QD (given 12 h apart) (treatment C). Each treatment was given for 7 days. On day 8, subjects were admitted to the General Clinical Research Center at UNC for intensive PK sampling. After the first PK visit, subjects crossed over to each of the other two treatments. Intensive PK sampling was performed at the end of each 7-day treatment.

Fosamprenavir (700-mg tablets [investigational during the study period] [GlaxoSmithKline, Research Triangle Park, NC]), lopinavir/ritonavir (Kaletra; 133/33-mg capsules [Abbott Laboratories, Abbott Park, IL]), and ritonavir (Norvir; 100-mg capsules [Abbott Laboratories, Abbott Park, IL]) were supplied by GlaxoSmithKline and used throughout the study.

The evening prior to PK sampling, study subjects were admitted to the Verne S. Caviness General Clinical Research Center at the University of North Carolina Hospitals. Evening doses of FPV, LPV/RTV, and additional RTV (if applicable) were observed and recorded. Adherence was assessed by pill counts and medication administration records. After an overnight fast, the morning dose of each medication was administered with a standardized meal, and the time was recorded. For the visits in which the doses were separated, FPV/RTV and LPV/RTV were given with standardized meals. The total daily caloric intake per visit was standardized to 2,000 to 2,500 cal, made up of 55% carbohydrates, 30% fat, and 15% protein.

Each PK sampling schedule varied in length depending on the treatment being investigated. For treatment A, blood samples were obtained at 0 h (predose) and 0.5, 1, 2, 4, 6, 8, and 12 h after the doses of FPV plus LPV/RTV. For treatment B, the blood sampling scheme was designed to characterize the full 12-hour PK profiles of both FPV/RTV and LPV/RTV (given 4 h after FPV/RTV), using the following times: 0 h (pre-FPV/RTV dose) and 0.5, 1, 2, 4 (pre-LPV/RTV dose), 4.5, 5, 6, 8, 10, 12, and 16 h after the FPV/RTV dose. Likewise, for treatment C, the blood sampling scheme was designed to characterize the full 24-h PK profiles of FPV/RTV and LPV/RTV, using the following times: 0 h (pre-FPV/RTV dose) and 0.5, 1, 2, 4, 6, 8, 12 (pre-LPV/RTV), 12.5, 13, 14, 16, 18, 20, 24, 30, and 36 h after the FPV/RTV dose. Whole blood was collected in sodium citrate (for APV and FPV)- or sodium heparin (for LPV and RTV)-containing Vacutainer tubes (Becton Dickinson). Blood tubes were inverted to ensure mixing of the anticoagulant and whole blood and were centrifuged at 2,000 rpm at 4°C within 30 min of collection. Plasma was aliquoted and stored at −70°C until analysis.

TABLE 1. Study design[a]

Group	Sample size (*n*)	Treatment during period:		
		1 (days 11–17)	2 (days 18–24)	3 (days 24–30)
1	2	A	B	C
2	2	B	C	A
3	2	C	A	B
4	1	A	C	B
5	2	B	A	C
6	2	C	B	A

[a] Lead-in phase, FPV at 700 mg BID plus LPV/RTV at 400/100 mg BID (given simultaneously) for 10 days; treatment A, FPV at 700 mg BID plus LPV/RTV at 400/100 mg BID (given simultaneously) for 7 days; treatment B, FPV/RTV at 700/100 mg BID plus LPV/RTV at 400/100 mg BID (given 4 hours prior to FPV/RTV) for 7 days; treatment C, FPV/RTV at 1,400/200 mg QD plus LPV/RTV at 800/200 mg QD (given 12 hours prior to FPV/RTV) for 7 days.

APV and FPV concentrations were measured by Advion Biosciences (Ithaca, NY). Briefly, 50 μl of blood plasma was subjected to solid-phase extraction followed by high-performance liquid chromatography–tandem mass spectrometry detection with a Turbo ion-spray interface and multiple reaction monitoring in the positive-ion mode. This method has lower limits of quantification of 10 ng/ml for amprenavir and 5 ng/ml for FPV. Linearity was demonstrated up to the higher limits of quantification of 10,000 ng/ml for amprenavir and 1,000 ng/ml for FPV. All samples were analyzed on the same day, with an intraday variability of <12% for FPV and <6% for APV.

LPV and RTV concentrations were measured by Covance Laboratories, Inc. (Madison, WI). Briefly, 100 μl of blood plasma was processed by solvent extraction. Extracts were analyzed by high-performance liquid chromatography–tandem mass spectrometry using atmospheric-pressure chemical ionization. This method has lower limits of quantification of 10 ng/ml for RTV and 20 ng/ml for LPV. Linearity was demonstrated up to the higher limits of quantification of 10,000 ng/ml for RTV and 20,000 ng/ml for LPV. All samples were analyzed on the same day, with an intraday variability of <14% for LPV and <13% for RTV.

Noncompartmental pharmacokinetic analysis was performed using WinNonLin Pro (V4.0.1; Pharsight, Mountain View, CA) software. The AUC over the dosing interval ($AUC_{0\text{-}\tau}$) was calculated using the linear-log trapezoidal rule. Additional PK parameters analyzed included the maximum concentration (C_{max}), time to maximum concentration (T_{max}), and concentration at the end of the dosing interval (C_τ). For treatments A and B, τ was defined as 12 h, and for treatment C, τ was defined as 24 h.

Adverse events were assessed by a questionnaire administered at each study visit. In addition, a clinician performed physical exams on all subjects at screening and at each study visit. Laboratory parameters obtained at baseline were evaluated at each study visit. The ACTG toxicity grading scale was used for toxicity documentation (2). Subjects experiencing grade 3 or 4 toxicity were reported to the UNC Institutional Review Board and subsequently withdrawn from the study. In addition, any subjects with the following elevations were withdrawn from the study: fasting cholesterol level of >280 mg/dl, triglyceride level of >500 mg/dl, blood glucose level of >120 mg/dl, and aspartate aminotransferase or alanine aminotransferase level of more than twice the upper limit of normal at any time during the study. Blood sampling was not conducted for any subject with a hematocrit of <30%.

The primary outcome measures for this study were the amprenavir $AUC_{0\text{-}\tau}$ and C_τ. The sample size was conservatively calculated using APV C_τ values, which are more variable than $AUC_{0\text{-}\tau}$ values (coefficients of variation, 43 and 29%, respectively) (4). Using this parameter, a sample size of 11 at a significance level of 0.05 was calculated to have >85% power to detect at least a 30% difference in means across the three levels of repeated measures. Secondary

TABLE 2. Steady-state PK parameters for APV, LPV, and RTV given in various dosing strategies[a]

Study drug and APV PK parameter	GM (95% CI)			GMR (95% CI)		
	A	B[b]	C[b]	B/A	C/A	C/B
APV						
C_{max} (μg/ml)	1.88 (1.47, 2.40)	1.24 (1.06, 1.44)	2.94 (1.98, 4.37)	0.66 (0.54, 0.80)	1.56 (1.02, 2.41)	2.37 (1.57, 3.58)
AUC_{τ} (μg · h/ml)	10.21 (7.99, 13.04)	6.82 (5.36, 8.69)		0.67 (0.54, 0.83)		
AUC_{24} (μg · h/ml)	20.42 (15.98, 26.08)	13.64* (10.72, 17.38)	15.67* (11.39, 21.55)		0.77 (0.59, 0.99)	1.15 (0.88, 1.50)
C_{τ} (μg/ml)	0.52 (0.37, 0.75)	0.27* (0.17, 0.41)	0.15* (0.08, 0.26)	0.51 (0.32, 0.81)	0.28 (0.19, 0.41)	0.55 (0.33, 0.92)
LPV						
C_{max} (μg/ml)	8.10 (6.03, 10.87)	12.98* (11.07, 15.21)	12.42* (9.09, 16.96)	1.60 (1.31, 1.95)	1.53 (1.20, 1.95)	0.96 (0.75, 1.22)
AUC_{τ} (μg · h/ml)	69.50 (46.26, 104.41)	122.48 (103.02, 145.63)		1.76 (1.34, 2.32)		
AUC_{24} (μg · h/ml)	139.00 (92.52, 208.82)	244.96* (206.04, 291.26)	199.18* (127.36, 311.50)		1.43 (1.02, 2.01)	0.81 (0.55, 1.20)
C_{τ} (μg/ml)	2.98 (1.51, 5.91)	8.08* (6.79, 9.60)	5.03 (2.10, 12.04)	2.71 (1.45, 5.05)	1.69 (0.97, 2.95)	0.62 (0.25, 1.53)
RTV						
C_{max} (μg/ml)	0.59 (0.40, 0.87)	2.83* (1.88, 4.26)	3.30* (2.25, 4.85)	4.84 (2.98, 7.86)	5.65 (4.06, 7.86)	1.17 (0.72, 1.90)
AUC_{τ} (μg · h/ml)	3.87 (2.73, 5.49)	20.50 (14.09, 29.84)		5.30 (3.66, 7.37)		
AUC_{24} (μg · h/ml)	7.74 (5.46, 10.98)	41.00* (28.18, 58.68)	34.43* (21.73, 54.55)		4.45 (3.09, 6.41)	0.84 (0.51, 1.37)
C_{τ} (μg/ml)	0.15 (0.10, 0.22)	1.36* (0.80, 2.32)	0.72* (0.33, 1.59)	9.26 (5.47, 15.68)	4.94 (2.44, 10.00)	0.53 (0.25, 1.14)

[a] Treatment A ($n = 11$), FPV at 700 mg BID plus LPV/RTV at 400/100 mg BID (given simultaneously) for 7 days; treatment B ($n = 11$), FPV/RTV at 700/100 mg BID plus LPV/RTV at 400/100 mg BID (given 4 hours prior to FPV/RTV) for 7 days; treatment C ($n = 11$), FPV/RTV at 1,400/200 mg QD plus LPV/RTV at 800/200 mg QD (given 12 hours prior to FPV/RTV) for 7 days. The AUC_{24} values for treatments A and B are based on 2× the observed AUC_{12}. *, $P < 0.02$ compared to treatment A.

[b] RTV daily dose was increased by 200 mg.

outcome measures were the lopinavir and ritonavir $AUC_{0\text{-}\tau}$ and C_{τ}. Summary statistics were generated for the following parameters for all drugs across all treatments: C_{τ}, $AUC_{0\text{-}\tau}$, C_{max}, and T_{max}. Geometric means (GMs), geometric mean ratios (GMRs), and their respective 95% confidence intervals (95% CI) for $AUC_{0\text{-}\tau}$ and C_{τ} values were calculated using SAS 8.2 software (SAS, Cary, NC). When twice-daily dosing was used, the $AUC_{0\text{-}24}$ was calculated by multiplying by 2. The primary analysis of amprenavir $AUC_{0\text{-}\tau}$ and C_{τ} values used a mixed model with a compound symmetry covariance structure, with adjustment for period effects. Demographic data are reported as means ± standard deviations (SD), while pharmacokinetic data are presented as geometric means with 95% CI.

RESULTS

A total of 15 healthy volunteers were enrolled in the study, and 11 completed all periods of the study, from January to June 2003. Four subjects withdrew prematurely due to adverse events, with three withdrawing during the first 10 days of the lead-in phase due to grade 2 rashes (considered drug related) and one withdrawing after 25 days due to leukocytosis and folliculitis (not considered drug related). Of the 11 subjects who completed the study, 10 were male, 9 were Caucasian, and 2 were African-American. These subjects had a mean (SD) age of 23 (±5.5) years and a mean (SD) weight of 81 (±12.87) kg.

Table 2 contains pharmacokinetic data for APV, LPV, and RTV for each treatment, along with treatment comparisons. Figure 1 shows graphical representations of the geometric mean concentration-time data for APV, LPV, and RTV across all three treatments.

Compared to those after simultaneous drug administration, the APV C_{τ} and $AUC_{0\text{-}\tau}$ when FPV/RTV was given 4 hours prior to LPV/RTV declined by 49% (95% CI, 19 to 68%; $P < 0.02$) and 33% (95% CI, 17 to 46%; $P < 0.02$), respectively, while the LPV C_{τ} and $AUC_{0\text{-}\tau}$ increased by 171% (95% CI, 45 to 405%; $P < 0.02$) and 76% (95% CI, 34 to 132%; $P < 0.02$), respectively.

Compared to those after simultaneous drug administration, the APV C_{τ} and $AUC_{0\text{-}\tau}$ when FPV/RTV was given 12 h prior to LPV/RTV declined by 72% (95% CI, 59 to 81%; $P < 0.02$) and 23% (95% CI, 1 to 41%; $P < 0.02$), respectively, while the LPV C_{τ} and $AUC_{0\text{-}\tau}$ increased by 69% (95% CI, −3 to 195%; $P = 0.10$) and 43% (95% CI, 2 to 101%; $P < 0.02$), respectively.

With dose separation, the total daily dose of RTV increased from 200 mg to 400 mg. Compared to those after simultaneous administration, the RTV C_{τ} and $AUC_{0\text{-}\tau}$ increased 826% (447 to 1,468%) and 430% (266 to 667%), respectively, with a 4-hour separation and 394% (144 to 900%) and 345% (209 to 541%), respectively, with a 12-hour separation.

To further understand the mechanism of the FPV-LPV/RTV interaction, plasma concentrations of FPV were quantified. Detectable FPV concentrations were found in only 5 of 407 samples, representing 4 of 11 subjects. These concentrations ranged from 6.12 to 8.43 ng/ml. There was no pattern in the detectable concentrations with respect to treatment phase or time within the dosing interval.

Adverse events, such as rash, gastrointestinal upset (diarrhea, loose stools, or nausea), and perioral paresthesias, were documented. Three of 15 subjects discontinued the study after 6, 7, and 9 days of simultaneous administration due to drug-related, grade 2 maculopapular rashes. In all cases, the rash resolved within 14 days after treatment with antihistamines. One patient withdrew during the last period of the study due to non-drug-related leukocytosis and folliculitis. These were successfully treated with a short course of minocycline and topical clindamycin. All subjects experienced loose stools for an average of 23.8 days, which resolved with ($n = 4$) or without ($n = 7$) antidiarrheal therapy (loperamide or atropine/diphenoxylate). Four subjects had grade 1 or 2 diarrhea for an average of 13 days, with three of the four having diarrhea only during treatments B and C, where an additional 200 mg of RTV was given daily. However, no subjects withdrew from the study due to gastrointestinal tract-related side effects. No subjects experienced diarrhea during their pharmacokinetic visits. Four subjects had taste perversion for 6 to 30 days, and two subjects had perioral paresthesia for 30 days.

Adherence to the study medication was acceptable through-

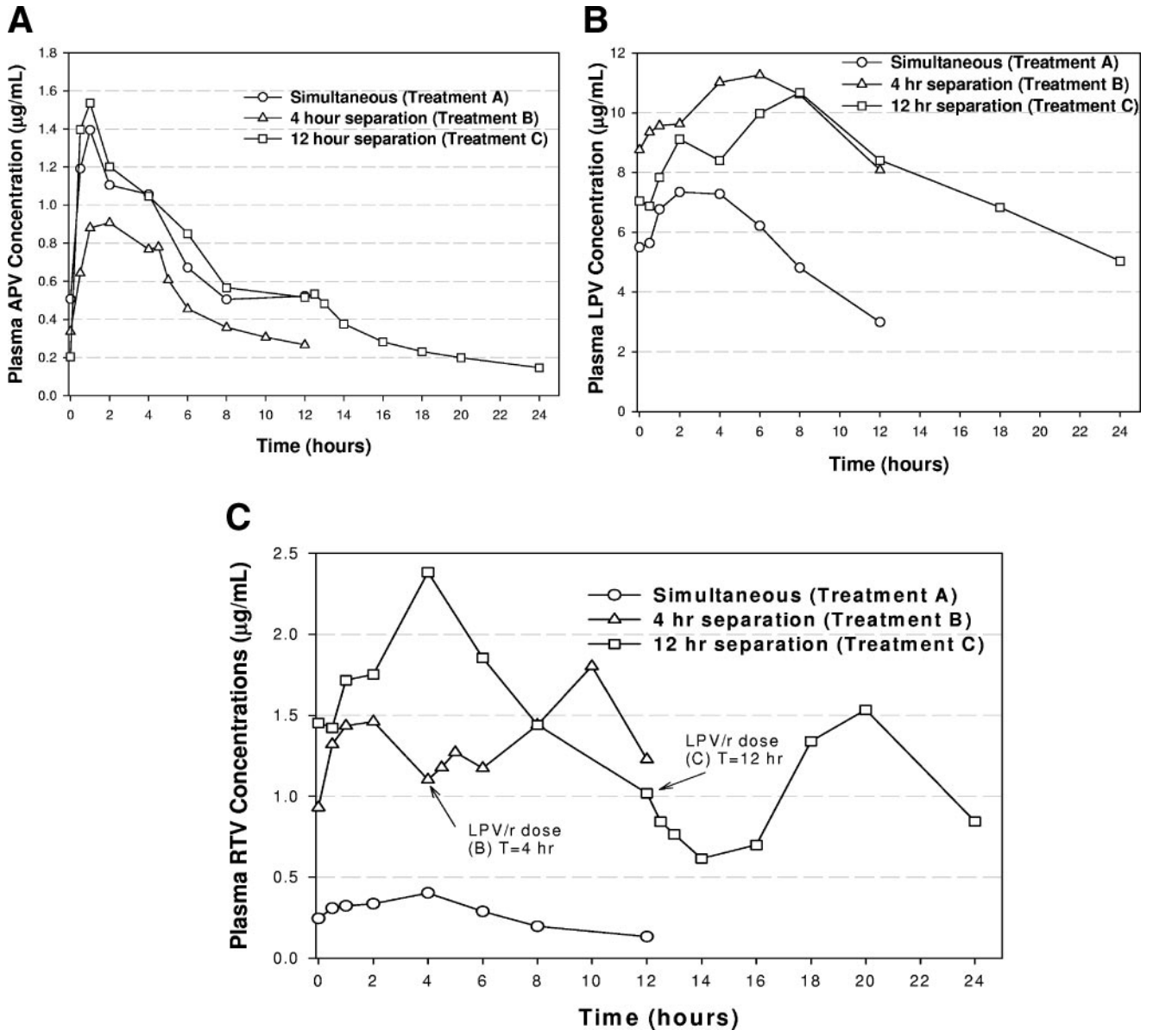

FIG. 1. Curves of geometric mean concentration versus time. Treatment A (n = 11), FPV at 700 mg BID plus LPV/RTV at 400/100 mg BID (given simultaneously) for 7 days; treatment B (n = 11), FPV/RTV at 700/100 mg BID plus LPV/RTV at 400/100 mg BID (given 4 h prior to FPV/RTV) for 7 days; treatment C (n = 11), FPV/RTV at 1,400/200 mg QD plus LPV/RTV at 800/200 mg QD (given 12 h prior to FPV/RTV) for 7 days. (A) Amprenavir curve; (B) lopinavir curve; (C) ritonavir curve.

out the investigation. By diary and pill counts, five subjects were 100% adherent and six subjects were 89 to 99% adherent to their study medication.

DISCUSSION

Drug interactions between protease inhibitors can be unpredictable. This is due in part to the complex interaction between cytochrome P450 (CYP) enzyme activity, cellular membrane transporter (e.g., p-glycoprotein) activity, and protein binding (18). Despite these complex interactions, dually boosted protease inhibitor regimens are commonly used therapies for HIV-infected patients with limited treatment options, although efficacy data are limited (6, 9, 19, 24).

The pharmacokinetics of the combination of APV (the Agenerase formulation) and LPV/RTV have been investigated in a number of publications (4, 19, 20, 26). Compared to the data for APV at 600 mg BID given with RTV at 100 mg BID, these data suggest that APV concentrations are decreased when it is coadministered with LPV/RTV at 400/100 mg BID. However, one study demonstrated comparable APV concentrations to those of historic controls (APV at 600 mg and RTV at 100 mg BID) when APV at 750 mg BID was coadministered with LPV/RTV (4).

Data from GlaxoSmithKline evaluating the interaction between fosamprenavir and LPV/RTV also demonstrated a decline in APV concentrations when it was given with LPV/RTV. Administering an extra 100 mg BID of RTV along with the standard doses of FPV and LPV/RTV resulted in lower FPV exposure than treatment with FPV/RTV alone (65% decrease in APV C_{τ} and 63% decrease in APV $AUC_{0\text{-}\tau}$). Doubling the FPV dose to 1,400 mg BID and increasing the doses of LPV/RTV to 533/133 mg BID also resulted in lower FPV exposure than that with FPV/RTV alone (42% decrease in APV C_{τ} and 26% decrease in APV $AUC_{0\text{-}\tau}$). However, both of these FPV/LPV/RTV combination regimens were poorly tolerated in this healthy volunteer population, with 31 to 33% of subjects discontinuing treatment due to adverse events, which were mostly gastrointestinal in nature.

Since the APV interaction cannot be overcome by increasing doses of FPV or RTV, it was hypothesized that a physical incompatibility between FPV and LPV/RTV or an acute local interaction involving drug-metabolizing enzymes and/or transporters may affect the absorption phase of the pharmacokinetic profile. A strategy of physical dose separation was pursued. While the 12-h dose separation strategy was studied for its practical application to patient therapy, the 4-h dosage separation strategy was pursued to examine whether a short physical separation could overcome any potential absorption interaction. Previously, this type of dosing strategy has been shown to overcome the effects of antacids on fluoroquinolone antibiotics, itraconazole, and protease inhibitors (3, 14, 23). Additionally, with the 4-h separation strategy, RTV was given simultaneously with FPV instead of relying on the RTV component of LPV/RTV to boost APV concentrations. This decision was based on data from ACTG study 378, in which optimal concentrations of saquinavir (SQV) were achieved when RTV was given simultaneously with SQV, in contrast to when RTV was given 4 h before or after SQV (28).

None of our dose separation strategies were able to avoid the effects of LPV/RTV on the APV concentration. In fact, when FPV and LPV/RTV were separated by 4 and 12 h, concentrations of APV were further decreased (the $AUC_{0\text{-}\tau}$ GMR [95% CI] for the 4-h strategy was 0.67 [0.54 to 0.83], and that for the 12-h strategy was 0.77 [0.59 to 0.99]), despite the fact that patients received twice the total daily dose of RTV. Since this investigation did not include subjects receiving FPV/RTV and LPV/RTV alone, we can only compare exposures in this study to historic data from healthy volunteers (29). APV concentrations in all treatment arms were substantially lower than historic data (geometric mean APV $AUC_{0\text{-}\tau}$, C_{τ}, and C_{max} for FPV/RTV at 700/100 mg BID, 36.5 h · μg/ml, 2.35 μg/ml, and 5.72 μg/ml, respectively) (29). LPV concentrations were lower than historic controls when given simultaneously with FPV (geometric mean LPV $AUC_{0\text{-}\tau}$, C_{τ}, and C_{max} for LPV/RTV at 400/100 mg BID, 92.6 h · μg/ml, 6.05 μg/ml, and 11.3 μg/ml, respectively). However, both the 4-h and 12-h strategies returned LPV exposure to values seen in HIV-infected patients and healthy volunteers using both twice-daily and once-daily regimens (4, 13, 29). We attribute this increase in LPV exposure to an increase in RTV dosing, similar to what was seen with other dosing strategies in healthy volunteers (29).

In this investigation, the exposure of APV when FPV and LPV/RTV were given in standard doses was lower than that reported for HIV-infected subjects (13). Therefore, it is possible that our results might differ from data from an HIV-infected patient population. However, reports of disparate PK data between HIV-infected patients and healthy volunteers are rare. Due to the extreme nature of this interaction and the higher drug exposure needed in an antiretroviral treatment-experienced patient population, it is unlikely that a dose separation strategy would be effective in these patients.

Additional considerations of a mechanism for this interaction include changes in alkaline phosphatase activity and/or protein binding interactions. Fosamprenavir is converted to APV at and/or in the intestinal epithelium by alkaline phosphatase (7). Although LPV has not been shown to affect alkaline phosphatase activity in vitro (GlaxoSmithKline, personal communication), other excipients in the Kaletra formulation have not been tested. In this investigation, detection of FPV in the plasma was sporadic, and no differences were seen between the dosing groups.

Protein binding displacement interactions between these protease inhibitors could result in lower total drug concentrations of APV, which would not be overcome with increased doses or dose separation. APV and LPV are >90% and >98% protein bound, respectively, and primarily bind to alpha-1-acid glycoprotein and albumin (1, 7). If LPV displaced APV from these proteins, a decrease in the total APV concentration (with no change in unbound/free drug APV concentrations) would be expected (5). This interaction would have no clinical consequences, since the unbound concentration represents the amount of drug available to exert antiviral activity. However, Taburet et al. noted a decline in both total and free APV concentrations in nine individuals given 400/100 mg LPV/RTV with 600 mg APV plus either 100 or 200 mg of RTV (as the Agenerase formulation). The decline in total drug concentration suggests a protein binding displacement of APV. The decline in unbound concentration suggests that additional mechanisms are likely to be involved, such as the induction of CYPs or other transporters (26).

Finally, it was previously observed that combining lopinavir with ritonavir in vitro leads to a 10-fold decrease in the CYP3A inhibitory potency of ritonavir (15). If there were a similar magnitude of discrepancy in ritonavir inhibition in patients, even doubling the dose of ritonavir would not be sufficient to achieve adequate inhibition of APV metabolism and increase drug concentrations to baseline.

The results of this study suggest that the interaction between FPV and LPV/RTV is neither a physicochemical incompatibility nor an acute effect on drug-metabolizing enzymes or transporters that can be overcome by separating or increasing drug doses. Further investigation into the mechanisms of this interaction are warranted, including looking into possible chronic induction effects on drug-metabolizing enzymes and/or transporters (10, 12). Alternate strategies need to be devised to allow effective coadministration of these antiretroviral agents.

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